85 What is bacterial colonisation of cystic fibrosis children toothbrushes?

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Objectives: *Pseudomonas aeruginosa* or *Staphylococcus aureus* toothbrushes contamination in cystic fibrosis (CF) patients is unknown. The objective of this pilot study was to determine prevalence of those germs on toothbrushes of CF and healthy children, and define if toothbrushes may be involved in pulmonary infection.
Methods: Toothbrushes and sputum bacteriological analysis from children between 8 and 18 year old was conducted: 27 CF patients, 15 healthy siblings and 15 healthy children out of patient family.
Results: *Staphylococcus aureus* has been identified on 23% toothbrushes from patients, and 13% of healthy children, without any methicillin-resistant specimen. *P. aeruginosa* was detected on 15% of patients toothbrushes, and 0% to 13% of healthy children. There was no statistical link between pulmonary colonisation and toothbrush contamination.
Conclusion: CF patient toothbrushes can be colonised by *S. aureus* or *P. aeruginosa.* Impact on pulmonary colonisation is still unknown. Toothbrush decontamination methods need to take into account these bacteria in CF patients.

86 Comparative study for the evaluation of a new technology for cystic fibrosis screening

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Objectives: Cystic fibrosis (CF) is one of the most frequently diagnosed autosomal-recessive diseases in the Caucasian population. Screening for Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene mutations, including poly T, is strongly recommended in infertile couples planning a pregnancy by assisted reproductive technology (ART). This study evaluated the performance of the new Nanochip CF70 kit (Savyon Diagnostic, Israel), a microarray assay, and compared it with the Innolipa kits (Innogenetics, Belgium).
Methods: From January to July 2012 we analyzed 392 blood samples with Innolipa and Nanochip technologies that identify respectively 70 and 56 CFTR mutations. Both tests include the most common Italian mutations and the poly-T screening. Discordant results were analyzed with the Devyser CFTR Core Kit (Devyser, AB, Sweden), MLPA (MRC Holland), Direct Sequencing (DS) on the 3730 DNA Analyzer (AppliedBiosystems), and Sequenom's MassArray system (Diatechpharmacogenetics, Italy).
Results: Innolipa and NanoChip were concordant for 371/392 samples. 21/392 (0.5%) discordant results were tested with the aforementioned technologies: DS confirmed Innolipa results in 18/21 samples and Nanochip results in 1/21, while Devyser and Sequenom did not recognize some mutations not included in their panels. DS was essential for the identification of two different homozygous deletions; although they were not present in Innolipa panels, in 2/21 samples Innolipa indicated a mutation with the warning no interpretation possible.
Conclusion: In this study the Innolipa assay confirmed its reliability and Nanochip showed that it could become competitive with slight changes to the software.

87 Metagenomic approach to bacterial identification in induced sputum from cystic fibrosis subjects

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Objectives: To apply the 16S rRNA pyrosequencing technique to identify bacterial species of induced sputum (IS).
Methods: A total of 62 IS samples were collected from unrelated clinically stable CF-patients [42 males, median age of 18.5 years ranged from 6 to 52, 21 of them F508del homozygous (33.4%) and 28 heterozygous (45.1%)] attended in three different CF-Units of Madrid, Spain (Hospital Ramón y Cajal n = 31, Hospital La Princesa n = 21, and Hospital La Paz n = 10). Total DNA was obtained with a manual protocol of extraction. Metagenomic profiles were determined using tag-encoded 16S rRNA gene and pyrosequenced by 454/FLX Titanium (Roche).
Results: From the 62 samples processed, 51 samples offered adequate quality results. The median of the bacterial loads was 2,748 copies per IS sample (ranged from 37 to 24,083). Taking account of all samples, the major bacterial *Phyla* detected were Proteobacteria (78.71%) [g-Proteobacteria 74.13%, b-Proteobacteria 4.42, d-Proteobacteria 0.13%, and α-Proteobacteria 0.02%]; Firmicutes (15.03%); Actinobacteria (3.23%); and finally Bacteroidetes (2.56%). In 41 subject, more than 50% of the total bacteria present in the sputum corresponded to the g-Proteobacteria Class, in which is included the genus *Pseudomonas*. Detection of uncultured or not-previously reported bacterium was anecdotic.
Conclusion: Metagenomic analysis of sputum samples corroborated that *Pseudomonas aeruginosa* (g-Proteobacteria) and *Staphylococcus aureus* (Firmicutes) are the major pathogens present in the lung of CF-subjects. Nevertheless, a great intra-individual diversity was found in both bacterial density and phylum's proportions.

88 Use of MALDI-TOF-MS for identification of anaerobic bacteria in CF patients

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Introduction: Cystic Fibrosis (CF) lung infection is polymicrobial, including fastidious and slow growing anaerobic bacteria, poorly identified by conventional methods. Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) is a powerful, rapid, precise and cost-effective method for bacteria identification.
Aim: To demonstrate that MALDI-TOF-MS could be a good option for identification of CF anaerobic bacteria.
Methods: CF respiratory samples were processed using both anaerobic and aerobic bacteriological technique. Isolated microorganisms were identified by MALDI-TOF-MS.
Results: 32 CF samples were collected from 32 CF patients (mean age 23 years, range 1–50). Anaerobes from 12 different species were detected in 69% of samples. The most representative bacteria belonged to *Prevotella* spp. (40%), *Veillonella* spp. (22%), *Gemella* spp. (12%). *Pseudomonas aeruginosa* (Pa) was isolated in 44% of CF studied patients.
Discussion: Despite of restricted number of samples, anaerobic flora is not age-related, furthermore anaerobic lung colonization seems not influenced by the concomitant Pa infection. Different studies demonstrated the use of MALDI-TOF-MS for routine identification of anaerobic bacteria. These organisms are poorly identified using phenotypic and molecular approaches. The extreme speed and low cost of MALDI-TOF-MS could improve laboratory efficiency of anaerobic bacteria identification, relevant for efficient treatment.